



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|--|---|--|
| <p>(51) International Patent Classification ⁶ : A61K 31/195, 31/21, 31/295, 31/34 // (A61K 31/195, 31:56, 31:57, 31:565, 31:00) (A61K 31/295, 31:56, 31:57, 31:565, 31:00) (A61K 31/21, 31:56, 31:57, 31:565, 31:00) (A61K 31/34, 31:56, 31:57, 31:565, 31:00)</p> | <p>A1</p> | <p>(11) International Publication Number: WO 97/25984</p> <p>(43) International Publication Date: 24 July 1997 (24.07.97)</p> |
| <p>(21) International Application Number: PCT/US97/00795</p> <p>(22) International Filing Date: 21 January 1997 (21.01.97)</p> <p>(30) Priority Data: 08/588,586 18 January 1996 (18.01.96) US</p> <p>(71) Applicants: SCHERING AKTIENGESELLSCHAFT [DE/DE]; Muellerstrasse 178, D-13342 Berlin (DE). BOARD OF REGENTS THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 West Seventh Street, Austin, TX 78701 (US).</p> <p>(72) Inventors: GARFIELD, Robert, E.; 1814 Winding Way, Friendswood, TX 77546 (US). CHWALISZ, Kristof; Lobbersteig 70, D-13503 Berlin (DE).</p> <p>(74) Agents: HAMLET-KING, Diana et al.; Millen, White, Zelano & Branigan, P.C., Arlington Courthouse Plaza I, Suite 1400, 2200 Clarendon Boulevard, Arlington, VA 22201 (US).</p> | <p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p> | |
| <p>(54) Title: TREATMENT OF URINARY INCONTINENCE WITH NITRIC OXIDE SYNTHASE SUBSTRATES AND/OR NITRIC OXIDE DONORS</p> <p>(57) Abstract</p> <p>The invention provides a method for the treatment and prevention of urinary incontinence in mammals, e.g., human males and females, especially nonpregnant female mammals, by administering a nitric oxide synthase substrate and/or nitric oxide donor, alone or in combination with an estrogenic agent and/or a progestational substance, with or without supplementation with an alpha-adrenergic agonist, beta-adrenergic receptor blocking agent, cholinergic-receptor blocking compound or a cholinergic-receptor-stimulating drug, as well as pharmaceutical compositions useful in practicing the methods of this invention.</p> | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|--|----|--------------------------|
| AM | Armenia | GB | United Kingdom | MW | Malawi |
| AT | Austria | GE | Georgia | MX | Mexico |
| AU | Australia | GN | Guinea | NE | Niger |
| BB | Barbados | GR | Greece | NL | Netherlands |
| BE | Belgium | HU | Hungary | NO | Norway |
| BF | Burkina Faso | IE | Ireland | NZ | New Zealand |
| BG | Bulgaria | IT | Italy | PL | Poland |
| BJ | Benin | JP | Japan | PT | Portugal |
| BR | Brazil | KE | Kenya | RO | Romania |
| BY | Belarus | KG | Kyrgyzstan | RU | Russian Federation |
| CA | Canada | KP | Democratic People's Republic of Korea | SD | Sudan |
| CF | Central African Republic | KR | Republic of Korea | SE | Sweden |
| CG | Congo | KZ | Kazakhstan | SG | Singapore |
| CH | Switzerland | LI | Liechtenstein | SI | Slovenia |
| CI | Côte d'Ivoire | LK | Sri Lanka | SK | Slovakia |
| CM | Cameroon | LR | Liberia | SN | Senegal |
| CN | China | LT | Lithuania | SZ | Swaziland |
| CS | Czechoslovakia | LU | Luxembourg | TD | Chad |
| CZ | Czech Republic | LV | Latvia | TG | Togo |
| DE | Germany | MC | Monaco | TJ | Tajikistan |
| DK | Denmark | MD | Republic of Moldova | TT | Trinidad and Tobago |
| EE | Estonia | MG | Madagascar | UA | Ukraine |
| ES | Spain | ML | Mali | UG | Uganda |
| FI | Finland | MN | Mongolia | US | United States of America |
| FR | France | MR | Mauritania | UZ | Uzbekistan |
| GA | Gabon | | | VN | Viet Nam |

TREATMENT OF URINARY INCONTINENCE WITH NITRIC OXIDE
SYNTHASE SUBSTRATES AND/OR NITRIC OXIDE DONORS

Background of the Invention

5 This invention relates to a method for the treatment and/or prevention of urinary incontinence usually in women during the post menopausal period or during pregnancy or postpartum with a nitric oxide synthase substrate (e.g., L-arginine), a nitric oxide donor or both, alone or
10 in combination with estrogen and/or progestin (e.g., hormone replacement therapy (HRT)), or with supplementation with alpha-adrenergic agonists, beta-adrenergic receptor blocking agents, cholinergic-receptor-blocking compounds and/or cholinergic-receptor-stimulating drugs.

 Women in industrialized nations can now expect to spend over a third of their lives in the postmenopausal period. One of the major problems women face during the climacteric period is urinary incontinence.
15 Urinary incontinence is also a problem during pregnancy or postpartum. In pregnancy, this condition may be related to altered steroid and nitric oxide levels rather than the physical presence and pressure of the growing fetus.

20 Urinary incontinence is the inability of the bladder to retain urine resulting in urine loss as a consequence of either urge (urge incontinence), or physical or mental stress (stress incontinence). There have been many studies of the effects of estrogen and progesterone therapy for incontinence (Barbieri, 1994; Sartori et al., 1995). These studies
25 indicate that estrogen and/or progesterone replacement therapy can par-

5 tially alleviate incontinence in some women (Elia and Bergman, 1993; Sartori et al, 1995). However, there is no conclusive evidence that hormone therapy alone is sufficient to cure incontinence (Cardozo and Kelleher, 1995). Some studies have shown that hormone replacement
10 therapy helps prevent postmenopausal recurrent urinary tract infections and improves urinary incontinence (Cardozo and Kelleher, 1994). Other studies suggest hormone supplementation with alpha-adrenergic agonists, beta-adrenergic-receptor blocking agents, cholinergic-receptor-blocking compounds and cholinergic-receptor-stimulating drugs (Barbieri, 9194; Brandeis and Resnick, 1992).

15 The normal bladder fills at a physiological rate dictated by the function of the kidneys and the bladder can accommodate large volumes of urine. This phenomenon has been attributed to physical properties of the bladder as well as a neural inhibitory system. The inhibitory mechanism may involve inhibition of parasympathetic activity
20 or an increase in sympathetic tone to produce detrusor relaxation and allow filling to occur. During filling the outlet neck of the bladder and urethra are contracted preventing leakage. Voiding or micturition is characterized by a relaxation of the outlet neck and the urethra followed
25 by contraction of the detrusor muscle. The process begins again when the bladder is empty and the detrusor relaxes and the outlet neck and urethra contract to seal off the bladder and maintain continence.

It is now well known that HRT (hormone replacement therapy), such as estrogen treatment, improves or reverses the adverse effects of
30 the decrease of sex steroid secretion by the ovaries during menopause. Estrogens have also been shown to improve mood and psychological well-being in postmenopausal women and they also prevent atrophic changes in the urogenital tract. Estrogens have been shown to effect arterial tone and this may help to explain the reduction in hot flushes
35 observed in postmenopausal women with estrogen therapy. On the other hand, unopposed estrogen therapy has been associated with endometrial hyperplasia and endometrial cancer. Many studies have shown that the addition of progesterone to estrogen HRT decreases the risk of endometrial cancer and even reverses endometrial hyperplasia. However, progestins are not without their own untoward side effects.

Progestins may reinforce the beneficial effects of estrogens on the cardiovascular system. Modern HRT now employs combinations of an estrogen and a progestin as in the general case for most contraceptives.

One of the most exciting recent advances in biology and medicine is the discovery that nitric oxide is produced by endothelial cells and that it is involved in the regulation of vascular tone, platelet aggregation, neurotransmission and immune activation (Furchgott and Zawadzki, 1980; Moncada, Palmer and Higgs, 1991; Ignarro, 1991). Nitric oxide is an important mediator of relaxation of the muscular smooth muscle (Montada, Palmer and Higgs, 1991) and was formerly known as EDRF (endothelin-derived relaxing factor) (Furchgott and Zawadzki, 1980; Moncada, Palmer and Higgs, 1991). Nitric oxide is synthesized by the oxidative deamination of a guanidino nitrogen of L-arginine by at least three different isoforms of a flavin-containing enzyme, nitric oxide synthase (Montada, Palmer and Higgs, 1991). Synthesis of nitric oxide has been shown to be competitively inhibited by analogues of L-arginine; NG-nitro-L-arginine methyl ester (L-NAME), NG-monoethyl-L-arginine (LMMA), N-iminoethyl-L-ornithine (L-NIO), L-monomethyl-L-arginine (L-NNMA) and L-NG-methylarginine (LNMA) and Nw-nitro-L-arginine (L-NA).

Nitric oxide elevates levels of cGMP (1,4,5-cyclic guanosine monophosphate) within the vascular smooth muscle to produce relaxation and to reduce blood vessels tone (Moncada, Palmer and Higgs, 1991). Nitric oxide binds to heme and thus activates soluble guanylate cyclase (Ignarro, 1991) to increase the cellular content of cGMP. It has long been recognized that nitrovasodilators, such as nitro-prusside and nitroglycerin, inhibit vascular smooth muscle contractility to produce relaxation or to reduce vascular tone. These agents have been used since the late 1980s as vasodilators. However, only recently has the mechanism of action of these compounds been realized. Nitrovasodilators are now classified as nitric oxide donors because they are metabolized or spontaneously release nitric oxide (Moncada, Palmer and Higgs, 1991). The long-used nitrovasodilators may be regarded as substitution therapy for a failing physiological mechanism. Nitric oxide is also produced by macrophages and other immune cells.

- 4 -

There is a substantial body of evidence from animal experiments that a deficiency in nitric oxide contributes to the pathogenesis of a number of diseases, including hypertension, atherosclerosis and diabetes (Montada, Palmer and Higgs, 1991). There are many recent studies showing that the inhibition of nitric oxide synthase dramatically increases blood pressure.

Nitric oxide may also be involved in accommodation of the bladder during filling or relaxation of the bladder neck and urethra during voiding. The bladder is innervated by nonadrenergic and noncholinergic nerves (NANC nerves) and nitric oxide is thought to be a neurotransmitter in these types of nerves (Ehren et al, 1994; Andersson and Persson, 1994; Smet, et al, 1994; Lee et al., 1994). There is evidence showing that nitric oxide containing nerves are localized to a greater extent in the outlet region and urethra compared to the detrusor (Andersson and Persson, 1994; Lee et al., 1994). However, whether nitric oxide is involved in voiding by relaxing the urethra and bladder neck or in bladder filling by relaxing the detrusor is unclear. There have been few studies of the effects of nitric oxide donors on either the urethra, bladder neck or detrusor muscle.

Summary of the Invention

Our studies suggest that nitric oxide donors effectively relax the detrusor muscle (see below). Further our studies of the uterus, cervix and vascular tissues suggest that the steroid hormones control nitric oxide synthesis, release and the effector system for nitric oxide (Chwalisz and Garfield, 1994). Therefore, we have discovered that the substrate for nitric oxide, nitric oxide donors, or both in combination with steroid hormones (estrogen and/or progesterone) are useful for treatment and prevention of urinary or urethra incontinence.

It is an object of the invention to provide a method for the treatment and prevention of urinary incontinence with a nitric oxide substrate and/or donor in mammals, e.g., human males and females, especially nonpregnant female mammals.

It is another object of the invention to provide a method for treatment and prevention of urinary incontinence with a nitric oxide substrate and/or donor in pregnant or postpartum female mammals.

5 It is a further object of the invention to provide a method for treatment and prevention of urinary incontinence with a nitric oxide substrate and/or donor in which an estrogenic agent in combination with a nitric oxide substrate and/or donor is used for urinary incontinence in both nonpregnant or pregnant female mammals.

10 It is a further object of the invention to provide a method for treatment and prevention of urinary incontinence with a nitric oxide substrate and/or donor in which a partial estrogenic agent (e.g. raloxifen) in combination with a nitric oxide substrate and/or donor is used for urinary incontinence in both nonpregnant or pregnant female mammals.

15 In another aspect of this invention, a progestational agent is used in combination with a nitric oxide substrate and/or nitric oxide donor for treatment and prevention of urinary incontinence in female mammals.

In a further aspect of this invention, an estrogen and a progestin are used in combination with a nitric oxide substrate and/or nitric oxide donor for the treatment and prevention of urinary incontinence in female mammals.

20

It is another object of this invention to provide a method for the treatment and prevention of urinary incontinence with a nitric oxide substrate and/or nitric oxide substrate alone or in combination with an estrogenic agent and/or a progestational substance, and with or without supplementation with an alpha-adrenergic agonist, beta-adrenergic receptor blocking agent, cholinergic-receptor blocking compound or a cholinergic-receptor-stimulating drug.

25

A further object is to provide pharmaceutical compositions useful in practicing the methods of this invention. Upon further study of the specification and appended claims, further aspects, objects and advantages of this invention will become apparent to those skilled in the art.

30

Thus, in a method aspect, this invention relates to a method of treating urinary incontinence in a mammal, e.g., nonpregnant or pregnant female mammal, which comprises administering to an individual manifesting the symptoms thereof one or both of a nitric oxide sub-

35

- 6 -

strate and a nitric oxide donor, alone or in combination with an estrogen or a progestin, or both, and with or without supplementation with an alpha-adrenergic agonist, a beta-adrenergic receptor blocking agent, a cholinergic-receptor blocking compound or a cholinergic-receptor stimulating drug, all in amounts effective to ameliorate the symptoms thereof; typically, the amount of the nitric oxide synthase substrate and nitric oxide donor or both is effective to increase urinary continence by raising the blood level of circulating L-arginine in a female to whom the composition is administered to at least 10 to 500 μ mole above the normally 50 to 1000 μ mole circulating levels, or to raise nitric oxide donor levels to about 10 nM to 100 μ M (micromolar), the amount of the estrogen being bioequivalent to approximately 2 mg per day of estradiol (e.g., Progy-nova, Schering), the amount of a partial estrogen being bioequivalent to approximately 1 - 200 mg per day of raloxifen, and the amount of the progestational agent administered being bioequivalent to 50 - 300 mg of injected progesterone.

In a product aspect, this invention relates to a pharmaceutical composition comprising at least one of a nitric oxide synthase substrate (L-arginine) and a nitric oxide donor (eg. sodium nitroprusside or glyceryl trinitrate), alone or in further combination with one or more of a estrogen and/or progestin with and without added supplements of an alpha-adrenergic agonist, a beta-adrenergic receptor blocking agent, a cholinergic receptor blocking compound or a cholinergic stimulating drugs with the amount of the nitric oxide synthase substrate, a nitric oxide donor or both per unit dosage being equivalent to either raise the blood level of circulating L-arginine to least 10 to 500 μ mole above the normally 50 to 1000 μ mole circulating or raise nitric oxide donor levels to about 10 nM to 100 μ M, the amount of the estrogen being bioequivalent to about 2 mg of estradiol (e.g., Progynova, Schering), the amount of a partial estrogen being bioequivalent to approximately 1 - 200 mg per day of raloxifen, with the amount of the progesterone bioequivalent to 50 to 300 mg of injected progesterone and the amount of the supplemental alpha-adrenergic agonist, beta-receptor blocking agent, cholinergic-receptor blocking compound or cholinergic stimulating drug as indicated below.

The methods of this invention treat urinary incontinence in a menopausal/postmenopausal, nonpregnant or pregnant, and/or postpartum female, who is manifesting the symptoms thereof.

5 Because the conditions of menopause/postmenopause, pregnancy or postpartum are produced or aggravated by subnormal nitric oxide synthesis, both nitric oxide synthase substrates, e.g., L-arginine and nitric oxide donors, e.g., sodium nitroprusside, nitroglycerin, glyceryl tri-
10 nitrate, SIN-1, isosorbid mononitrate, isosorbid dinitrate and diethylene-triamine/NO (DETA/NO), are useful for ameliorating the symptoms thereof and, in one respect of this method of this invention, a combination of both are employed.

An additive effect is achieved when an estrogenic agent is administered concurrently with a nitric oxide substrate and/or nitric oxide donor. In the case of a female mammal, an estrogen can be admini-
15 stered concurrently with or in lieu of a progestin. The latter can also be used alone.

An additional effect is achieved when a nitric oxide substrate or a nitric oxide donor is administered either with estrogen or progestin and supplemented with one of a alpha-adrenergic agonist, a beta receptor
20 blocking agent, a cholinergic-receptor blocking agent or a cholinergic stimulating drug.

Thus, the method aspects of this invention and the pharmaceutical composition aspects of this invention employ either or both of a nitric oxide substrate and a nitric oxide donor and, optionally one or
25 more of, e.g., an estrogen (e.g., Prodynova, Schering) or a progestin (e.g., progesterone or norgestrel), with or without one of the following: an alpha-adrenergic agonist, a beta-receptor blocking agent, a cholinergic-receptor blocking compound or a cholinergic stimulating drug.

30 Examples of dosage ranges of typical NO-substrates and NO-donors (per os or transdermally) are:

- 8 -

| | | |
|---|-----------------------|--------------------------------------|
| | | total dose: |
| | L-Arginine | 500 mg - 10 g p.o. |
| | Sodium Nitroprusside | range 500 - 2000 μ g/kg/day p.o. |
| | Nitroglycerin | 0.5 - 10 mg p.o. |
| 5 | Nitroglycerin | 0.1 - 10 mg/24 hours transdermal |
| | Isosorbid mononitrate | 10 - 100 mg/day p.o. |
| | Isosorbid dinitrate | 10 - 100 mg/mg p.o. |

10 The nitric oxide donors (e.g., nitroglycerin) can be administered preferentially by a transdermal patch (e.g., Deponit 5/10/T [Schwarz Pharma], Nitroderm TTS 5/Nitroderm TTS 10 [CIBA]), orally (e.g., Corangin [CIBA], Nitrolingual forte or mitte [Pohl],) etc.

15 Examples of combinations of active agents which can be administered concurrently with a nitric oxide substrate and/or nitric oxide donor are the following estrogens and progestins and typical oral dosage ranges of the active agents of the estrogen and progestin type with the nitric oxide substrate or donor:

20 **Estrogens:** A daily dose bioequivalent to about 1-2 mg per day estradiol, e.g., Premarin, Wyeth-Ayerst, 0.625 mg/day; estradiol valerate, 50 μ g/day transdermally; vaginal estradiol creams, 1.25 mg/day and vaginal estradiol rings, 0.2 mg/day and the natural occurring estrogens used in hormone replacement therapy currently available.

25 **Partial estrogen agonists (partial estrogens):** A daily dose bioequivalent to about 1-200 mg per day, e.g. raloxifen ([6-hydroxy-2-(4-hydroxyphenyl)-3-benzothienyl][4-[2-(1-piperidinyl)ethoxy]phenyl]-methanon-hydrochloride), tamoxifen ((Z)-N,N-dimethyl-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]ethanamine, nafoxidin (1-[2-[4-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthalinyl)phenoxy]ethyl]pyrrolidin-hydrochloride), Mer-25 (a-[4-[2-(diethylamino)ethoxy]phenyl]-4-methoxy-a-phenyl-benzenethanol) and centchroman ((3R-trans)-3,4-dihydro-2,2-dimethyl-7-methoxy-3-phenyl-4-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]-2H-1-benzopyran).

30

- 9 -

5 **Progestins:** A daily dose bioequivalent to 50 - 300 mg of progesterone/day, e.g., an injectable suspension of medroxyprogesterone acetate to provide a weekly dose thereof of 100 - 1000 mg or tablets or dragees providing an oral dose thereof of 5 -100 mg/day, an injectable solution of hydroxyprogesterone caproate which provides a weekly dose of 250 - 500 mg; tablets, capsules or dragees of norethindrone acetate which provide a daily dose of 5 - 20 mg.

Examples of estrogen and progestin combinations are listed below:

| | | | |
|----|-------------------------|-----------------------------|--------------------------|
| 10 | Product | Composition | Dose (mg per day) |
| | Climaval (Sandoz) | Estradiol valerate | 1 or 2 |
| | Progynova (Schering) | Estradiol valerate | 1 or 2 |
| | Harmogen (Abbott) | Piperazine estrone | 1.05 or 2.5 |
| | Hormonin (Shire) | Estradiol | 0.6 |
| 15 | | + Estrone | |
| | | + Estriol | |
| | Premarin (Wyeth-Ayerst) | Conjugated equine Estrogens | 0.625, 1.25 or 2.5 |

20 Commercially available combination calendar packs for hormone replacement therapy include "Estrapak", "Prempak-C", "Trisequens", "Trisenquens forte" and "Cycloprogynova". The following are illustrative compositions of such products:

| | |
|----|--|
| | Estradiol 50 mg per day (28 days, 8 patches) |
| | conjugated equine estrogens 0.625 mg per day (28 days) |
| 25 | Estradiol valerate 2 mg per day (11 days) |
| | Estradiol valerate 2 mg per day |
| | Norgestrel 0.5 mg per day (10 days) |
| | Norgestrel 0.15 mg per day (12 days) |
| | conjugated equine Estrogens 1.25 mg per day (28 days) |

- 10 -

Norgestrel 0.15 mg per day (12 days)
 estradiol 2 mg per day + estriol 1 mg per day (22 days)

5 Norethisterone acetate 1 mg per day (10 days)
 estradiol 1 mg per day + estriol 0.5 mg per day (6 days)
 estradiol 4 mg per day + estriol 2 mg per day (21 days)

10 Norethisterone acetate 1 mg per day (10 days)
 Estradiol 1 mg per day + estriol 0.5 mg per day (6 days)
 Estradiol valerate 1 mg per day (21 days)

Levonorgestrel 0.25 mg per day (10 days)
 Estradiol valerate 2 mg per day (21 days)

Levonorgestrel 0.5 mg per day (10 days)

Daily doses of progestogens taken for 12 days per month in
 patients receiving oral or transdermal estrogens:

| | | |
|----|-----------------------------|----------------------|
| 15 | Norethisterone | 0.7 - 2.5 mg per day |
| | Medroxyprogesterone acetate | 10 mg per day |
| | Norgestrel | 150 μ g per day |
| | Dydrogesterone | 10 - 20 mg per day |

20 Typical dosages of exemplary supplemental agents include those
 shown below, other bioequivalent amounts of analogous such agents
 being routinely determinable:

Alpha-adrenergic-receptor-agonists:

Phenylpropanolamine 25 - 100 mg daily

Phenylephrine 5 - 15 mg daily

Beta-receptor-blocking agents:

| | |
|----|-------------------------------|
| 25 | Propranolol 20 - 120 mg daily |
| | Befaxolol 10 - 40 mg daily |
| | Acebutolol 400 mg daily |
| | Atenolol 50 - 100 mg daily |
| | Bisoprolol 5 - 10 mg daily |

- 11 -

Cholinergic-receptor blocking compounds:

Benztropine 0.5 - 1 mg daily

Biperiden 3 - 6 mg daily

Propantheline 30 - 120 mg daily

5 Cholinergic-stimulating drugs:

Bethanecol 30 - 120 mg daily

Many other examples of compounds in each of the four foregoing categories are well known and can be employed in this invention.

10 The pharmacologically active agents employed in this invention can be administered in admixture with conventional excipients, i.e., pharmaceutically acceptable liquid, semi-liquid or solid organic or inorganic carriers suitable, e.g., for parental or enteral application and which do not deleteriously react with the active compound in admixture there-
15 with. Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions, alcohols, vegetable oils, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycer-
20 ides, pentaerythritol fatty acid esters, hydroxy methylcellulose, polyvinyl pyrrolidone, etc.

20 The pharmaceutical preparations can be sterilized and if desired mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure; buf-
fers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds.

25 For parental application, particularly suitable are solutions, preferably oily or aqueous solutions, as well as suspensions, emulsions, or im-
plants, including suppositories, transdermal patches, and vaginal gels, creams and foams. Ampoules are convenient unit dosages.

30 In a preferred aspect, the composition of this invention is adapted for ingestion.

For enteral application, particularly suitable are unit dosage forms, e.g., tablets, dragees or capsules having talc and/or carbohydrate carrier or binder or the like, the carrier preferably being lactose and/or corn starch and/or potato starch; particulate solids, e.g., granules; and liquids

and semi-liquids, e.g., syrups and elixirs or the like, wherein a sweetened vehicle is employed. Sustained release compositions can be formulated including those wherein the active compound is protected with differentially degradable coatings, e.g., by microencapsulation, multiple coatings, etc.

Suitable for oral administration are, inter alia, tablets, dragees, capsules, pills, granules, suspensions and solutions. Each unit dose, e.g., each tablespoon of liquid or each tablet, or dragee contains, for example, 5 - 5000 mg of each active agent.

Solutions for parenteral administration contain, e.g., 0.01 - 1% of each active agent in an aqueous or alcoholic solution.

The nitric oxide substrate and/or donor can be administered as an admixture with an estrogen and/or progestational agent and/or any other optional active agent or as a separate unit dosage form, either simultaneously therewith or at different times during the day from each other.

The combination of active agents is preferably administered at least once daily (unless administered in a dosage form which delivers the active agents continuously) and more preferably several times daily, e.g., in 2 to 6 divided doses. The typical dose is about 0.5 to 1000 mg of each active agent, although some less active agents, e.g., L-Arginine, require much higher oral dosages, e.g., 500 to 10,000 mg, and others, e.g., sodium nitroprusside, require lower doses, e.g., 500 - 2,000 $\mu\text{g/kg/day}$. Doses for nitroglycerine typically are orally 2.6 mg 2 x daily; sublingually, 0.8 mg 1 - 4 x daily; and transdermally, 0.2 - 0.5 mg/hr. Since the LD_{50} dosages of most of these active agents is known in the prior art, a lower dosage regimen can be initiated and the dosage increased until a positive effect is achieved or a higher dosage regimen can initially be employed, e.g., in a crisis situation, and the dosages regulated downward as relief from the symptoms is achieved. Combinations of agents can be employed either continuously or sequentially.

In humans, both L-arginine and progesterone (or bioequivalent of another progestin) should be given in a ratio which produces blood plasma levels of 50 - 5000 μmolar L-arginine, 30 - 100 nmolar progesterone and 500 to 1000 nmolar of estradiol.

Brief Description of the Drawings

Various other objects, features and attendant advantages of the present invention will be more fully appreciated as the same becomes better understood when considered in conjunction with the accompanying drawings, in which like reference characters designate the same or similar parts throughout the several views, and wherein:

Figure 1: Figure 1 shows the effects of sodium nitroprusside (SNP) (10^{-3} M) on contractions of the rat detrusor muscle after stimulating the muscle with methylcholine (MC at 10^{-6} M and 10^{-5} M).

Figure 2: Data similar to Figure 1 is demonstrated in Figure 2.

Figure 3: Effects of DETA/NO (diethylenetriamine/NO) a nitric oxide donor compound on spontaneous contractions of the rat detrusor muscle.

Detailed Description of the Invention

In the experiments whose results are shown in Figure 1, rat bladder detrusor muscle was obtained from ovariectomized and normal non-pregnant animals. The tissues were suspended in muscle baths to record *in vitro* contractility and drugs were added to the baths to estimate their effects on the mechanical events. In the experiments shown in Figures 1 and 2, methylcholine (MC at 10^{-6} M and 10^{-5} M) was added to precontract the bladder samples, then the nitric oxide donor sodium nitroprusside (SNP at 10^{-3} M) was added to the bath. SNP caused an immediate and significant decrease in contractility. The relaxation response was transient which is typical of SNP for this preparation. Similar data was obtained from 16 other strips of tissues suspended *in vitro* and treated with SNP.

In the results shown in Figure 3, tissues were contracting spontaneously *in vitro*. When DETA/NO, a nitric oxide donor compound, was added to the muscle bath, spontaneous contractions abruptly disappeared and did not reappear during the recording period, about 30 minutes. Similar results were obtained from tissues from 16 rats. Additionally, tissues from ovariectomized rats treated with estrogen showed more pronounced effects than untreated ovariectomized rats ($n = 8$).

It can be concluded from these results that nitric oxide has a profound relaxation effect on the rat detrusor muscle. Since L-arginine is the substrate for nitric oxide one can deduce that nitric oxide substrates will also relax detrusor muscle. Further, since the effects of nitric oxide are much greater after treating rats with estrogen it can be concluded that estrogen plus a nitric oxide donor or a nitric oxide substrate may have greater effects when an nitric oxide substrate or donor are combined with estrogen. Furthermore, since estrogen and progesterone often act synergistically one can infer that estrogen and/or progesterone combinations would be useful. Relaxation of the detrusor muscle with nitric oxide donors indicates that nitric oxide may be involved in detrusor relaxation during the filling phase of bladder function. Therefore, nitric oxide donors and/or substrates alone or in combination with steroids will prove effective for urinary incontinence. Furthermore, since the bladder is innervated by adrenergic and cholinergic nerves combinations with alpha adrenergic agonists, beta-receptor blocking agents, cholinergic-blocking compounds or cholinergic stimulating drugs will be useful to treat incontinence.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative and not limitative of the remainder of the disclosure in any way whatsoever.

In the foregoing and in the following examples, all temperatures are set forth uncorrected in degrees Celsius; and, unless otherwise indicated, all parts and percentages are by weight.

The entire disclosures of all applications, patents and publications, cited above and below, if any, are hereby incorporated by reference.

EXAMPLES

Example 1: Treatment of urinary incontinence

5 To a nonpregnant human female (ca 60 years; 50 - 90 kg) displaying the signs of menopause or postmenopausal symptoms, including amenorrhea, and urinary incontinence, administer L-arginine 0.5 to 20 g of L-arginine per os daily in three divided doses until the symptoms are ameliorated. Thereafter, administer 0.5 to 5 g of L-arginine daily.

Example 2: Treatment of urinary incontinence

10 To a female comparable to and displaying the same symptoms as Example 1, administer daily 5 - 10 mg of nitroglycerine transdermally.

Example 3: Treatment of urinary incontinence

To a female comparable to and displaying the same symptoms as Example 1, administer daily 2 x 2.5 mg of nitroglycerine orally.

Example 4: Treatment of urinary incontinence

15 To a female similar to and displaying the same symptoms as Example 1, administer daily 0.5 to 20 g of L-arginine in combination with estrogen (e.g., estradiol valerate) 1 - 2 mg daily.

Example 5: Treatment of urinary incontinence

20 To a female similar to and displaying the same symptoms as Example 1, administer daily 2 x 5 mg nitroglycerine transdermally in combination with a partial estrogen agonist (e.g., raloxifen) 100 mg daily.

Example 6: Treatment of urinary incontinence

25 To a female similar to and displaying the same symptoms as Example 1, administer daily 0.5 to 20 g of L-arginine in combination with a partial estrogen agonist (e.g., raloxifen) 100 mg daily.

Example 7: Treatment of urinary incontinence

To a female similar to and displaying the same symptoms as Example 1, administer daily 2 x 2.5 mg nitroglycerin with a progestin (e.g., norgestrel) 150 µg per day.

Example 8: Treatment of urinary incontinence

To a female comparable to and displaying the same symptoms as Example 1, administer L-arginine 0.5 to 20 g daily and/or a nitric oxide donor (e.g., nitroglycerine, 2 x 2.5 mg) daily with or without one of the following, an estrogen (e.g., estradiol valerate) 1 - 2 mg daily, on a progestin (e.g., norgestrel, at 150 mg per day). The latter sex steroids to be given either continuously with L-arginine and/or a nitric oxide donor, or sequentially - the progestins taken for only 6 - 12 days per month.

Example 9: Treatment of urinary incontinence

To a female comparable to and displaying the same symptoms of Example 1, administer L-arginine (0.5 to 20 g daily) and/or a nitric oxide donor (e.g. nitroglycerine, 2 x 2.5 mg daily) with or without one or more of the following, an estrogen (e.g., estradiol valerate, 1 - 2 mg daily) or a progesterone (e.g. norgestrel, at 150 mg per day), an alpha-adrenergic agonist (e.g. phenylpropanolamine, 25 to 100 mg daily), a beta-receptor blocking agent (e.g. propranolol 20 - 120 mg daily), a cholinergic receptor blocking compound (e.g. propantheline 30 - 120 mg daily) or a cholinergic stimulating drug (e.g. bethanecol 30 - 120 mg daily).

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

REFERENCES

1. Barbieri, R.L. The bladder in menopause: Lower urinary tract dysfunction during the climacteric. *Curr. Problems Obstet. Gynecol. Fertil.* 1994; 17(6):196-228.
- 5 2. Eli, G. and Bergman, A. Estrogen effects on the urethra: beneficial effects in women with genuine stress incontinence. *Obstet. Gynecol.* 1993; 48(7):509-517.
- 10 3. Sartori, M.G., Baracat, E.C., Girad, M.J., Goncalves, W.J., Sartori, J.P., de Lima, G.R. Menopausal genuine stress urinary incontinence treated with conjugated estrogens plus progestogens. *Int. J. Gynecol. Obstet.* 1995; 49(2):165-169.
- 15 4. Cardozo, L.D. and Kelleher, C.J. Sex hormones, the menopause and urinary problems. *Gynecol. Endocrinol.* 1995; 9(1):75-84.
- 15 5. Cardozo, L. and Kelleher, C. Sex hormones and the female lower urinary tract. *Physiotherapy* 1994; 80:135-138.
- 20 6. Brandeis, G.H. and Resnick, N.M. Pharmacotherapy of urinary incontinence in the elderly. *Drug Therapy* 1992; 22:93-102.
- 20 7. Furchgott, R.F. and Zawadzki, J.V. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; 288:373-376.
- 25 8. Moncada, S., Palmer, R.M.G. and Higgs, E.A. Nitric oxide; physiology, pathophysiology and pharmacology. *Pharmacol. Rev.* 1991; 43:109-142.
- 25 9. Ignarro, L.J. Physiological significance of Nitric oxide. *Seminars in Perinatology* 1991; 15:20-26.
- 30 10. Ehren, I., Adolfsson, J. and Wilund, N.P. Nitric oxide synthase activity in the human urogenital tract. *Urol. Res.* 1994; 22:287-290.
- 30 11. Andersson, K.E. and Persson, K. Nitric oxide synthase and nitric oxide mediated effects in lower urinary tract smooth muscles. *World J. Urol.* 1994; 12:274-280.
- 35 12. Smet, P.J., Edyvane, K.A., Jonavicius, J., Marshall, V.R. Distribution of NADPH-diaphorase-positive nerves supplying the human urinary bladder. *J. Autonomic Nervous System* 1994; 47:109-113.
- 35 13. Lee, J.G., Wein, A.J., Levin, R.M. Comparative pharmacology of the male and female rabbit bladder neck and urethra: Involvement of nitric oxide. *Pharmacology*
- 40 14. Chwalisz, K. and Garfield, R.E. Role of progesterone during pregnancy: Models of parturition and preeclampsia. *Z. Geburtsh. u. Perinat.* 198:170-180.

WHAT IS CLAIMED IS:

1. A method of treating urinary incontinence symptoms in a female mammal, comprising administering to an afflicted female an effective amount of
 - (a) a nitric oxide synthase substrate, a nitric oxide donor, or both,and, optionally, further administering an effective amount of
 - (b) one or more of a progestin, an estrogen and a partial estrogen agonist.
2. The method of claim 1, wherein the mammal is a non-pregnant human female suffering from urinary incontinence.
3. The method of claim 1, wherein the mammal is a non-pregnant human female who has exhibited or is a candidate for hormone replacement therapy.
4. The method of claim 1, wherein the mammal is a non-pregnant female human and a nitric oxide synthase substrate is administered thereto.
5. The method of claim 1, wherein the mammal is a pregnant or postpartum human female.
6. The method of claim 1, wherein the nitric oxide substrate is L-arginine.

7. The method of claim 1, wherein the mammal is a non-pregnant human female and a nitric oxide donor is administered hereto.

8. The method of claim 1, wherein the nitric oxide donor is sodium nitroprusside, nitroglycerin, glyceryl trinitrate, SIN-1, isosorbid mononitrate or isosorbid dinitrate.

9. The method of claim 8, wherein the nitric oxide donor is administered transdermally.

10. The method of claim 8, wherein the nitric oxide donor is administered orally.

11. The method of claim 1, wherein the mammal is a non-pregnant human female and the nitric oxide substrate or donor is administered thereto in combination with an estrogen.

12. The method of claim 11, wherein the estrogen is estradiol valerate, conjugated equine estrogens, 17β -estradiol, estrone or estriol.

13. The method of claim 1, wherein the mammal is a non-pregnant human female and the nitric oxide substrate or donor is administered thereto in combination with a partial estrogen agonist.

14. The method of claim 13, wherein the partial estrogen agonist is raloxifen, centchroman or tamoxifen.

15. The method of claim 1, wherein the mammal is a non-pregnant human female and the nitric oxide substrate or donor is administered thereto in combination with a progestin.

16. The method of claim 15, wherein the progestin is progesterone, dydrogesterone, medroxyprogesterone, norethisterone, levonorgestrel, norgestrel, gestodene or drospirenone.

- 20 -

17. The method of claim 1, wherein the mammal is a non-pregnant human female and concurrently a hormone replacement amount of an estrogen or a progestin is administered thereto continuously.

18. The method of claim 1, wherein the mammal is a non-pregnant human female and concurrently hormone replacement amounts of an estrogen and a progestin are administered sequentially.

19. The method of claim 1, wherein the mammal is a human female and the nitric oxide substrate or donor is administered thereto in further combination with one or more of an alpha-adrenergic agonist, a beta-receptor blocking agent, a cholinergic-blocking compound or a cholinergic stimulating drug.

20. The method of claim 19, where in the mammal is administered estrogen and/or progestin with an alpha-adrenergic agonist, a beta-receptor blocking agent, a cholinergic-blocking compound or a cholinergic stimulating drug.

21. The method of claim 1, wherein the amount of a nitric oxide synthase substrate, a nitric oxide donor, or both is effective to raise the blood level of circulating L-arginine to at least about 50 - 5000 μ molar above the normally 50 - 1000 μ molar circulating levels or raise nitric oxide donor levels to about 10 nM to 100 μ M.

22. A pharmaceutical composition comprising an admixture of effective amounts of

- (a) a nitric oxide synthesis substrate, a nitric oxide donor or both, and
- (c) one or more of an alpha-adrenergic agonist, a beta-receptor blocking agent, a cholinergic blocking compound or a cholinergic stimulating drug, and, optionally,
- (b) an estrogen, a progestin or both.

- 21 -

23. The composition of claim 22, wherein (a) comprises a nitric oxide synthesis substrate.

24. The composition of claim 23, wherein the nitric oxide synthesis substrate is L-arginine.

25. The composition of claim 22, wherein (a) comprises a nitric oxide donor.

26. The composition of claim 25, wherein the nitric oxide donor is sodium nitroprusside, nitroglycerin, glyceryl trinitrate, SIN-1, isosorbid mononitrate or isosorbid dinitrate.

27. The composition of claim 22, wherein (b) comprises an estrogen, which is estradiol valerate.

28. The composition of claim 22, wherein (b) comprises a progestin which is levonorgestrel.

29. The composition of claim 22, wherein the amounts of (a), (c) and optionally (b) are effective to ameliorate the symptoms of urinary incontinence in a menopausal or postmenopausal female mammal.

30. The composition of claim 22, wherein the effective amount of (a) comprises an amount of the nitric oxide synthase substrate, nitric oxide donor or both effective to raise the blood level of circulating L-arginine to at least about 50 - 5000 μ molar above the normally 50 - 1000 μ molar circulating levels or raise the nitric oxide donor levels to about 10 nM to 100 μ molar, and the effective amount of (b) comprises an amount of estrogen equivalent to 1 - 2 mg of estradiol, an amount of progestin bioequivalent to 50 - 300 mg of injected progesterone, or both.

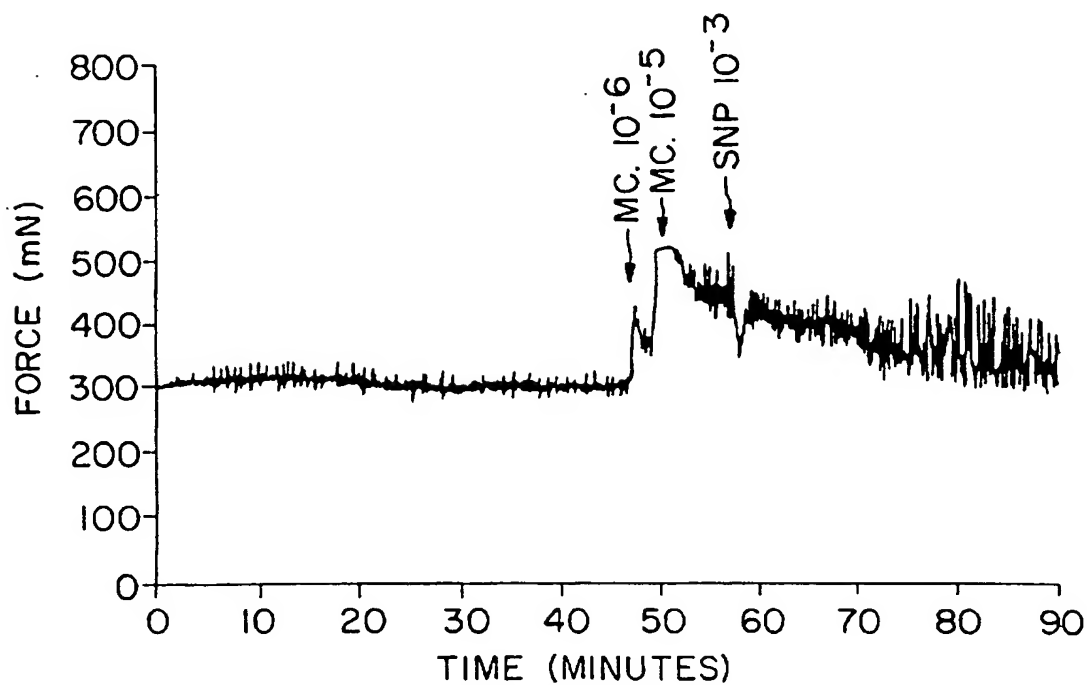


FIG. 1

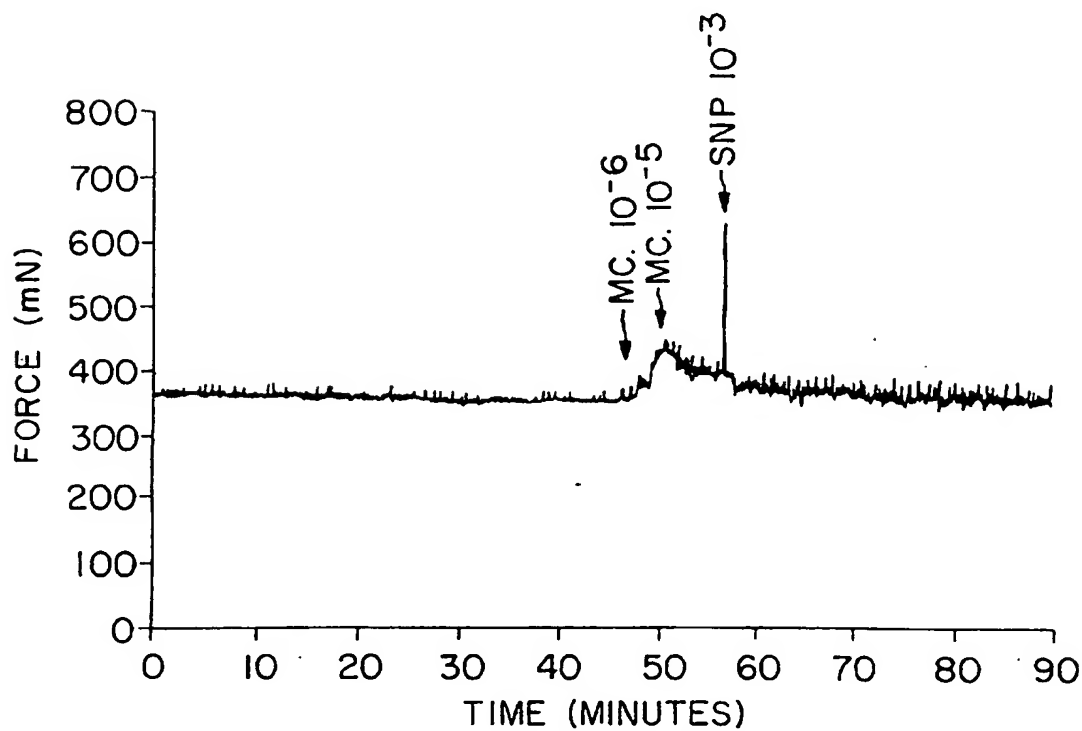


FIG. 2

2 / 2

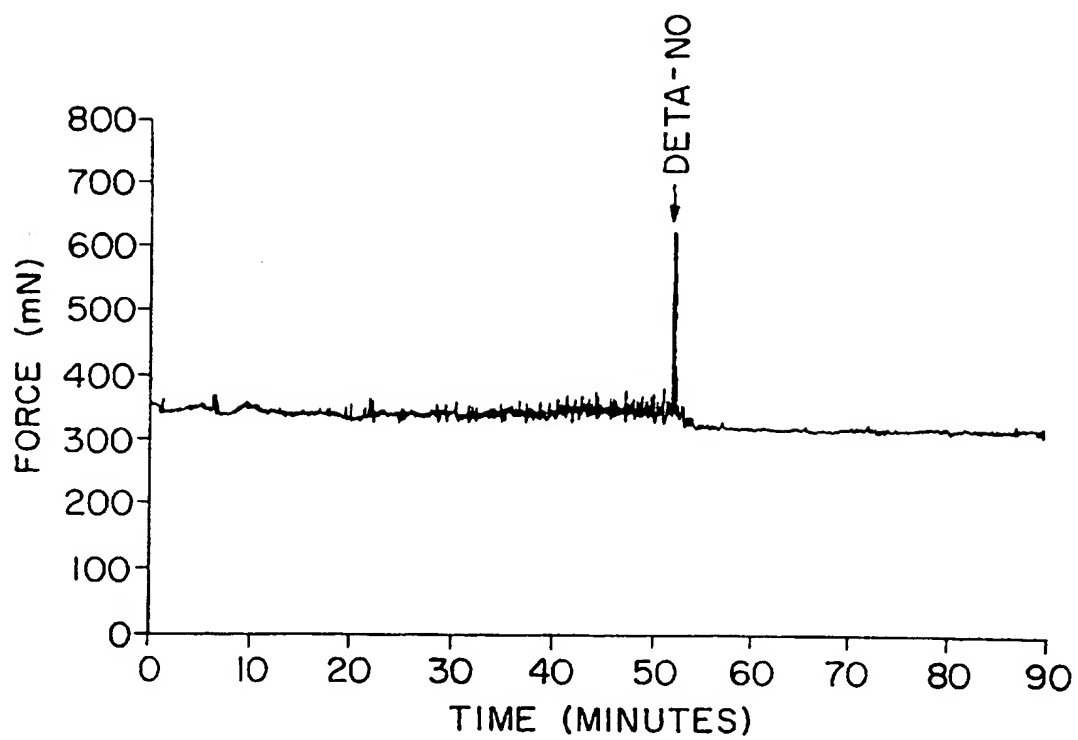


FIG. 3

INTERNATIONAL SEARCH REPORT

 Int'l Application No
 PCT/US 97/00795

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 A61K31/195 A61K31/21 A61K31/295 A61K31/34
 //(A61K31/195,31:56,31:57,31:565,31:00),(A61K31/295,31:56,31:57,
 31:565,31:00),(A61K31/21,31:56,31:57,31:565,31:00),(A61K31/34,

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------------|
| X | WO 95 13802 A (SCHERING AG ;GARFIELD ROBERT E (US); YALLAMPALLI CHANDRA (US)) 26 May 1995 see whole document, especially page 16 third paragraph, page 4 third paragraph, pages 5-7,11,14-16 and claims 1,2,8-10,12,21 --- | 1-12,15, 16, 19-26,30 |
| X | WO 95 13800 A (SCHERING AG ;YALLAMPALLI CHANDRA (US); GARFIELD ROBERT E (US)) 26 May 1995 see the whole document --- | 22-30 |
| X | WO 95 06466 A (KOREN LAB PTY LIMITED ;LUBOWSKI DAVID (AU)) 9 March 1995 see whole document, especially pages 4-5 --- -/-- | 22-26 |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" - document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

16 May 1997

Date of mailing of the international search report

29.05.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax (+31-70) 340-3016

Authorized officer

Gac, G

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US97/00795

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 31:56,31:57,31:565,31:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| A | <p>WORLD JOURNAL OF UROLOGY, vol. 12, no. 15, 1994, pages 274-280, XP000670769 ANDERSSON K -E ET AL: "NITRIC OXIDE SYNTHASE AND NITRIC OXIDE-MEDIATED EFFECTS IN LOWER URINARY TRACT SMOOTH MUSCLES" cited in the application see whole document, especially page 275 left column, page 277 left column, and page 278 right column lines 1-2 --- -/--</p> | 1,2,4-8 |

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents:

- * "A" document defining the general state of the art which is not considered to be of particular relevance
- * "E" earlier document but published on or after the international filing date
- * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- * "O" document referring to an oral disclosure, use, exhibition or other means
- * "P" document published prior to the international filing date but later than the priority date claimed

* "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

* "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

* "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

* "&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

29.05.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/US97/00795

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|---|-----------------------------|
| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| A | JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, vol. 265, no. 2, May 1993, pages 713-719, XP000647930 PARLANI M ET AL: "NONADRENERGIC, NONCHOLINERGIC INHIBITORY CONTROL OF THE RAT EXTERNAL URETHRAL SPHINCTER: INVOLVEMENT OF NITRIC OXIDE" see page 173 --- | 1,2,4-8 |
| A | SCANDINAVIAN JOURNAL OF UROLOGY AND NEPHROLOGY, no. SUPPL. 175, 1995, pages 43-53, XP000670055 ANDERSSON K -E ET AL: "NITRIC OXIDE SYNTHASE AND THE LOWER URINARY TRACT: POSSIBLE IMPLICATIONS FOR PHYSIOLOGY AND PATHOPHYSIOLOGY" see whole document, especially page 45 right column, page 46 left column and pages 47-50 --- | 1-4,6-8, 11,19, 22-26 |
| A | UROLOGY, vol. 45, no. 6, June 1995, pages 1071-1078, XP000670038 BURNETT ET AL.: "Nitric oxide control of lower genitourinary tract functions : a review" see page 1078, right-hand column see page 1075 - page 1076 see page 1012; table 1 --- | 1,4,6 |
| A | UROLOGY, vol. 44, no. 5, November 1994, pages 683-687, XP000670050 EHREN I ET AL: "LOCALIZATION OF NITRIC OXIDE SYNTHASE ACTIVITY IN THE HUMAN LOWER URINARY TRACT AND ITS CORRELATION WITH NEUROEFFECTOR RESPONSES" see the whole document --- | 1,4,6 |
| A | LIFE SCIENCES, vol. 48, no. 25, 1991, pages 2429-2436, XP000670051 DOKITA S ET AL: "NG-NITRO-L-ARGININE INHIBITS NON-ADRENERGIC, NON-CHOLINERGIC RELAXATION IN RABBIT URETHRAL SMOOTH MUSCLE" see the whole document --- -/-- | 1,4,6,8, 19,22-26 |

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US97/00795

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|---|----------------------------|
| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| A | J. UROLOGY, vol. 153, no. 6, June 1995, pages 2004-2009, XP000670037 BENNETT ET AL.: "Neural control of urethral outlet activity in-vivo : role of nitric oxide " see the whole document --- | 1,6 |
| A | CURR. PROBLEMS OBST. GYNECO. FERTIL., vol. 17, no. 6, November 1994 - December 1994, pages 196-229, XP000670162 UNKNOWN: "The bladder in menopause : lower urinary tract dysfunction during the climacteric" cited in the application see page 205 - page 207 see page 220 - page 224 --- | 3,11-20 |
| A | PHYSIOTHERAPY, vol. 80, no. 3, March 1994, pages 135-138, XP000670049 CARDOZO L ET AL: "SEX HORMONES AND THE FEMALE LOWER URINARY TRACT" cited in the application see the whole document --- | 1-3, 11-18, 22,27-30 |
| A | DRUG THERAPY, vol. 22, 1992, pages 93-96, 101/102, XP000670056 BRANDEIS G H ET AL: "PHARMACOTHERAPY OF URINARY INCONTINENCE IN THE ELDERLY" cited in the application see the whole document ----- | 3,11, 17-20 |

INTERNATIONAL SEARCH REPORT

In .ational application No.

PCT/US 97/00795

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-21
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/00795

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|---|--|
| WO 9513802 A | 26-05-95 | AU 8144594 A BR 9408067 A CA 2176721 A CN 1135178 A CZ 9601390 A EP 0730448 A FI 962109 A NO 961993 A PL 314465 A SK 63396 A | 06-06-95 24-12-96 26-05-95 06-11-96 16-10-96 11-09-96 15-07-96 12-07-96 16-09-96 05-03-97 |
| WO 9513800 A | 26-05-95 | US 5595970 A AU 8144694 A BR 9408062 A CA 2176727 A CN 1135177 A CZ 9601400 A EP 0730445 A FI 962110 A NO 961994 A PL 314466 A SK 63496 A | 21-01-97 06-06-95 24-11-96 26-05-95 06-11-96 11-09-96 11-09-96 15-07-96 16-07-96 16-09-96 05-03-97 |
| WO 9506466 A | 09-03-95 | AU 7454594 A ZA 9500511 A | 22-03-95 11-03-96 |